Chapter 2

Analysis of Grooming Behavior and Its Utility in Studying Animal Stress, Anxiety, and Depression

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Abstract

In rodents, grooming is a complex and ethologically rich behavior, sensitive to stress and various genetic and pharmacological manipulations, all of which may alter its gross activity and patterning. Observational analysis of grooming activity and its microstructure may serve as a useful measure of stress and anxiety in both wild and laboratory animals. Few studies have looked at grooming behavior more than cursorily, though in-depth analysis of the behavior would immensely benefit fields utilizing rodent research. Here, we present a qualitative approach to grooming activity and patterning analysis in mice, which provides insight into the effects of stress, anxiety, and depression on this behavioral domain. The method involves quantification of the transitions between different stages of grooming, the percentages of incorrect or incomplete grooming bouts, as well as the regional distribution of grooming activity. Using grooming patterning as a behavioral endpoint, this approach permits assessment of stress levels of individual animals, allows identification of grooming phenotypes in various mouse strains, and has vast implications in biological psychiatry, including psychopharmacology, genetics, neurophysiology, and experimental modeling of affective disorders.

Key words: Grooming behavior, stress, anxiety, depression, behavioral organization (sequencing), animal experimental and genetic models, neuropsychiatric disorders.

1. Background and Historical Overview

Grooming is an important and evolutionarily ancient behavior observed across many animal taxa *(1–4)*. Beyond the primary purpose of hygiene and caring for the body surface, grooming serves a variety of other functions, including stimulation of the skin, thermoregulation, chemo-communication, social interaction, de-arousal, and stress reduction *(1, 4–7)*. In both wild and laboratory rodents, this behavior constitutes 15–50% of waking

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time and may be triggered by novelty, swimming, pain, exposure to predators, or sexual behavior (for review *see (8, 9)*). Genetic factors play an important role in the regulation of rodent grooming, and various genetic manipulations have been reported to produce robust grooming phenotypes in mice *(6, 10–14)*.

Rodent grooming is a complex patterned behavior, which generally proceeds in a cephalocaudal direction *(3, 15, 16)*. The behavioral sequence (**Fig. 2.1**) usually begins with licking of the paws, followed by washing the nose and face, the head, the body, the legs, and finally washing and licking the tail and genitals *(3, 15, 16)*. Stereotyped grooming behaviors are clearly centrally controlled (rather than driven by peripheral sensory input), since mice with amputated front paws continued to make facial grooming gestures with their stumps *(5)*. Regulation of grooming behavior is mediated by multiple brain regions (especially the basal ganglia and hypothalamus) *(15–18)*, as well as by various endogenous agents (neuromediators *(5, 16, 19)*, hormones *(5, 20–23)*), and psychotropic drugs *(12, 19, 24–27)*. Given the robust nature of grooming behavior in animal phenotypes *(2, 9, 28, 29)*, it is logical to expect that alterations in this domain will be seen in experimental mouse models of stress, anxiety, and depression.

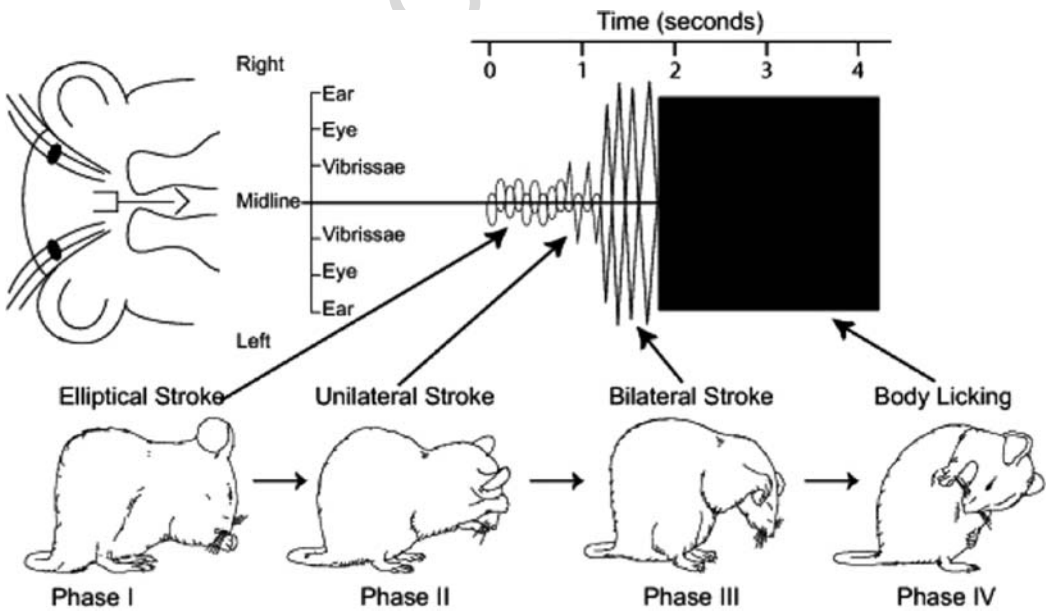

Fig. 2.1. Prototypical syntactic grooming chain pattern in mice (Prof. K. Berridge, with permission). Phase I*:* series of ellipse-shaped strokes tightly around the nose (paw, nose grooming). Phase II*:* series of unilateral strokes (each made by one paw) that reach up the mystacial vibrissae to below the eye (face grooming). Phase III*:* series of bilateral strokes made by both paws simultaneously. Paws reach back and upwards, ascending usually high enough to pass over the ears (head grooming). Phase IV*:* body licking, preceded by postural cephalocaudal transition from paw/head grooming to body grooming.

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Despite the complexity and importance of grooming in mice, many studies that include grooming observations have dealt with this behavior only cursorily. For example, some analyses include only cumulative grooming scores, or have lumped grooming into "overall activity scores" (for review *see (8, 9, 30)*). Furthermore, traditional measures of grooming often include only time to onset and/or the number and duration of bouts (**Table 2.1**), but ignore the unique, data-dense feature of this behavior – its complex microstructure *(27, 29, 30)*.

Table 2.1
Methodological approaches to mouse grooming phenotyping

Global assessment
• Coat state *(40–42)*
General cumulative measures
• The latency to onset, the duration, and the number of grooming episodes (bouts) *(28, 30)* • Temporal patterning (e.g., per-minute distribution) of grooming duration and frequency may be recorded to examine habituation of this behavior • The following patterns can be recorded for each bout: paw licking; nose/face grooming; head washing; body and leg grooming/scratching; tail/genitals grooming • Additional cumulative indices: the average duration of a single grooming bout, total number of transitions between grooming stages, and average number of transitions per bout *(8, 9)*
Patterning (sequencing)
• The percentages of incorrect transitions, as well as interrupted and incomplete grooming bouts *(8, 9, 30)*
Regional distribution of grooming
• Can be assessed as directed to the following five anatomic areas: forepaws, head, body, hind legs, and tail/genitals • Rostral grooming includes forepaw (preliminary rostral grooming) and head grooming. Body, legs, and tail/genital grooming can be considered as caudal grooming • Each bout can be categorized as being directed to (i) multiple regions or (ii) a single region, and the percentages of grooming bouts and of time spent grooming can be calculated for both categories *(6, 8, 9, 28, 30)*
Additional useful indices of grooming
• Probability of chain initiation (frequency of chain initiation per minute of grooming time) • Probability of pattern completion once initiated [these indices were not discussed here, but see *(8, 15, 16)* for details and useful background information]

Representing a typical displacement behavior, grooming is often seen in animal models of stress and anxiety *(19, 28, 31, 32)*, leading to a long-standing view of grooming as a mere anxiogenic response *(25, 33–35)*. Some data, however, indicate that higher stress or anxiety in animals does not necessarily translate into their

increased grooming activity *(27, 36–38)*. Such over-simplification of complex behavior has also been recently challenged by more detailed analyses of animal grooming phenotypes. Indeed, since grooming activity in rodents is increased under conditions of both high and low stress, the amount of grooming may not be a reliable indicator of animal anxiety *(8, 9, 27–30)*. However, unlike quantitative measures, the "quality" of grooming – its sequencing (**Table 2.1**) – varies substantially according to the degree of stress experienced *(8, 27, 30)*.

Specifically, low-stress "comfort" grooming occurs spontaneously as a transition between rest and activity, and generally proceeds in a "relaxed" uninterrupted manner following the cephalocaudal rule (**Fig. 2.1**). Conversely, stress-evoked grooming is generally characterized by frequent bouts of interrupted "chaotic" activity that defies the cephalocaudal rule, and may serve as a way to cope with fear or anxiety *(3, 30)*. Additionally, several manipulations (including brain lesions, psychotropic drugs, and genetic mutations) alter the behavioral microstructure of grooming *(8)*, sometimes without affecting the cumulative amount of grooming activity *(27)*. Therefore, traditional observations of grooming that focus only on quantitative measures of its activity (**Table 2.1**) are insufficient for proper interpretation of stress data, as they may provide ambiguous results *(9, 28, 30)*.

Alterations in the rodent depression-like states have also been shown to affect animal grooming *(39–42)*. However, unlike the "acute" nature of anxiety-induced grooming responses, the effects of depression on grooming are delayed and somewhat less obvious. Therefore, the role of grooming as a behavioral marker of depression has been much less studied, compared to the large body of literature on grooming responses to anxiety (see above). Do depressed animals groom more or less? Is the patterning of rodent grooming affected in depressed animals? Do these behavioral alterations in mouse grooming resemble clinical endophenotypes seen in depressed patients? These are the important questions that are currently under investigation, as they are only partially answered by the available literature on this topic, which will be briefly discussed further.

Because grooming represents only one domain, other behavioral endpoints and domains should be considered while performing an in-depth ethological analysis. However, the ability of grooming patterning to reflect (and indirectly measure) stress in mice has numerous potential applications. These include gauging the degree of stress induced by various tests, behavioral phenotyping of mutant or transgenic strains, and testing of psychotropic drugs for their ability to alter anxiety or depression levels *(9, 19, 30, 43)*. In addition, it

may assist in interpreting various non-grooming behaviors, and detect motor/coordination anomalies and age-related behavioral changes.

Furthermore, understanding ethological patterning of grooming also has implications for developing better mouse models of human behavioral disorders (such as obsessive-compulsive disorder, Rett or Tourette's syndrome), and for decoding normal human nervous behaviors elicited by everyday stress *(7, 8, 16, 44)*. This chapter will provide a detailed up-to-date overview of how researchers can assess mouse self-grooming behavior, and apply their findings to understand animal and human affective disorders.

2. Equipment, Materials, and Setup

Although various inbred, selectively bred, and genetically modified (mutant or transgenic) mice may be used to assess grooming *(28, 32, 43, 45, 46)*, in behavioral experiments, it is important to select the appropriate laboratory mouse strain. While some searchable online databases (such as Mouse Genome Informatics, MGI) may provide appropriate genetic models for studying mouse grooming, note that the activity fluctuates between strains and may be confounded by strain-specific phenotypes *(28, 30)* and other factors alike (see further).

In order to analyze animal grooming activity, transparent observation apparatuses (such as small plexiglas or glass boxes and cylinders) are generally utilized. For mouse studies, the dimensions of the apparatus may be $20 \times 20 \times 30$cm (although other dimensions may be used, depending on mouse activity and anxiety levels). Between sessions, it is necessary to remove olfactory cues in the apparatuses by thoroughly cleansing the equipment (e.g., with a 30% ethanol solution).

Researchers may also use various anxiolytic, anxiogenic, antidepressant, psychostimulant, and other psychotropic drugs to analyze their effects on grooming behaviors in mice. Common routes of injection include systemic [intraperitoneal (i.p.), intramuscular (i.m.), intravenous (i.v.), per oral (p.o.), subcutaneous (s.c.)] and local [intracerebral (i.c.) or intracerebroventricular (i.c.v)]. Route of administration, dose, and pre-treatment time generally vary depending on the drug and strain sensitivity. Importantly, all experimental procedures (including handling, housing, husbandry, and drug treatment) must be conducted in accordance with National and Institutional Guidelines for the Care and Use of Laboratory Animals.

3. Procedures

3.1. Coat State

Coat state assessment is the simplest method to evaluate animal grooming activity *(41, 47)*. After removing animals from their homecages, the state of the coat of eight separate body parts such as head, neck, forepaws, dorsal coat, ventral coat, hindlegs, tail, and genital region of each individual mouse may be inspected visually and recorded systematically *(40–42)*. For example, a score of 0 could be attributed to a coat in good form, and a score of 1 could be given to a dirty or disheveled coat. The resulting score (to be compared between different experimental groups) will represent the average for all body areas. Other similar scales may be used consistently within the laboratory to record the condition of the coat. Although this approach cannot be used to study acute effects of stress and anxiety, it has been shown that mouse coat state generally correlates with the level of experimental depression. For example, chronically stressed depressed mice generally display poor coat status, whereas antidepressant treatments tend to reverse this phenotype *(40–42)*. Thus, the coat state assessment provides a gross method of grooming analysis, and may reveal some very overt differences in animal behavior. Nevertheless, this method may lack ethological sensitivity, and therefore may need to be complemented with more sophisticated analyses of animal grooming that will be discussed further.

3.2. Acute Stress-Evoked Grooming

It is important to distinguish two forms of self-grooming in rodents: spontaneous (stress-evoked) and artificial grooming. To encourage stress-evoked grooming, a typical experiment may include exposure to novelty, such as a novel observation box, for 5–10 min. To ensure proper acclimation to the experimental room, it is recommended that rodents are transferred to the room at least 1 h before testing. The mouse may then be removed from the cage and presented with an anxiogenic stressor to stimulate grooming activity. In addition to novelty stress, researchers may also use stronger stressors, such as a brief pre-exposing the mouse to a bright light, conspecific, a predator (e.g., rat or cat) or its odor. In general, this procedure enables fast and reliable detection of alterations in mouse grooming related to anxiety domain, and may be a useful tool in basic research of emotionality.

3.3. Chronic Stress-Evoked Grooming

While chronic mild stress has been shown to reduce animal grooming *(40–42, 48)* (but *see (39)*), stronger stressors (such as olfactobulbectomy or peripheral anosmia), when applied chronically, produce pronounced activation of stereotypic

grooming activity. This "pathological" grooming is usually focused on a specific body area, and is accompanied by severe depression-like behaviors including anhedonia, hypoactivity, aggression, and self-aggression *(49–52)*. Overall, these procedures may be particularly relevant to modeling severe protracted depression in animals, and are generally in line with clinical data showing overall increases in stereotypic behavior (e.g., grooming disorders, hair-pulling) in depressed patients *(53, 54)*. However, more research is needed to understand whether animal depression produces consistent alterations in grooming patterning.

3.4. Artificial Grooming

Artificially induced grooming can be stimulated by allowing the mouse to swim or by smearing the animal with food *(8)*. The splash test is another method to evoke "artificial" grooming in mice. For this, a sucrose solution (e.g., 10%) may be squirted onto the mice in the dorsal region while they remain in their homecages *(40–42)*, and grooming activity measures (**Table 2.1**) can be recorded for 5 min after the vaporization of the solution. Misting with water (e.g., using fine water spray) is also an easy and reliable method to evoke artificial grooming behavior *(6, 30, 55)*, and is widely used in neurobehavioral experiments. Since spontaneous and artificial grooming represent two different forms of this behavior, abnormalities in one type do not necessarily imply deficits in another form of grooming. Thus, a parallel assessment of the two types of grooming is necessary for a more careful characterization of animal behavioral phenotypes *(6, 30, 56)*.

3.5. Hybridizing Behavioral Protocols

In addition to the above-mentioned procedures, researchers may consider combining several behavioral tests into a "smart battery" that simultaneously examines anxiety, depression, and grooming domains. For example, an initial 5-min open-field testing (to assess baseline anxiety and spontaneous novelty-induced grooming behavior) may be followed by the Porsolt's forced-swim test that evaluates depression-related immobility or despair *(57)*. In order to maximize the number of behavioral endpoints and domains per experiment, immediately after the forced-swim test, researchers may place the mice in an observation cylinder (e.g., for 5 min) to investigate artificial, swim-induced grooming *(43)*. Comparing the patterning and activity of the artificial post-swim grooming with the spontaneous (novelty-evoked) pre-swim grooming could lead to interesting findings regarding the animal's grooming phenotypes. In some instances, mice may also have a fatigability phenotype *(43, 57)* that should be discriminated from grooming behaviors. Fatigability will often interfere with mouse grooming activities, could confound data, and therefore needs to be carefully dissected from grooming domains (see further).

3.6. Time Required

To minimize initial procedure-related anxiety, researchers may choose to gently handle naïve mice 5 min/mouse/day for 3–4 days prior to the grooming experiments. Acclimation to the procedure room requires at least 1 h. The time required for grooming assessment protocols varies depending on the test battery used (see above), the number of animals per group and the number of experimental groups, and based on mouse grooming activity levels (see troubleshooting). In general, grooming behavior assessment will last 5–10 min per animal. Depending on the amount of grooming and other behavioral data collected, analysis could take between 2 and 4 days. It is advised that researchers maintain a 7-day minimum acclimation period between tests.

3.7. Data Analysis

To analyze the data, researchers may generally use the Mann–Whitney *U*-test for comparing two groups (parametric Student's *t*-test may be used if data are normally distributed) or an analysis of variance (ANOVA) for multiple groups, followed by a post hoc test. More complex designs, such as one-way ANOVA with repeated measures (time) or *n*-way ANOVA (additional factors: treatment, genotype, stress, sex, etc.), can also be used in grooming studies.

4. Experimental Variables

The present protocol, largely based on the method called the Grooming Analysis Algorithm (GAA) *(9)*, provides a high-throughput approach to analyze mouse grooming activity and microstructure. Several indices of grooming can be recorded as generalized measures, including coat state, latency to onset, cumulative duration of grooming, and number of bouts (grooming episodes); see **Table 2.1** for details. A shorter latency period to begin grooming, a longer duration of grooming, and more bouts may be behavioral markers for stress in mice (but see the discussion of validity of cumulative measures above). Calculating the average duration of a single bout (total time grooming/number of bouts), the total number of transitions between bouts, and the average number of transitions per bout (total number of transitions per bout/number of bouts) will also help provide necessary data in determining the level of stress of the mice.

To accurately evaluate grooming bout patterns, the researchers may develop a standardized scale to represent specific grooming activity and use it consistently within each laboratory. A typical scale may be as follows (**Table 2.1**): no grooming (0),

paw licking (1), nose, face, and head wash, characterized by pawing nose and semicircular strokes of the head and ears (2), body grooming, including body fur licking and scratching with hind paws (3), leg licking (4), and tail or genital grooming (5) *(8)*. However, researchers may modify this scale to suit their individual needs by including additional strain-specific grooming behaviors of interest, or by simplifying this scale for better detectability.

A "correct" bout is cephalocaudal in direction and follows a (0-1) (1-2) (2-3) (3-4) (4-5) (5-0) pattern of correct transitions (**Table 2.1**). An "incorrect" transition can vary from the model in one of four ways: an aborted or prematurely terminated bout (2-0, 4-0), a skipped transition (1-3, 2-5), a reversed bout (4-3, 5-2), or an incorrectly initiated bout (0-2, 0-5). A "complete" bout consists of a strict (0-1-2-3-4-5-0) sequence and any other pattern is considered incomplete. Frequently, researchers will notice grooming interruptions. Any sequence that contains at least one interruption is deemed "interrupted." However, an interruption of 6 s or greater is judged to be an entirely separate bout *(8, 9)*. Again, maintaining a consistent standard of all defined behaviors and criteria used within each laboratory is strongly recommended to avoid confusion and poor validity of data.

With this system, researchers may assess the three primary ethological measures of grooming patterning – the percentage of incorrect transitions, interrupted bouts, and incomplete bouts. In addition, researchers may calculate the duration of correct versus incorrect patterns, the number of interruptions during bouts, and the duration of complete versus incomplete bouts.

It is also useful to investigate the regional distribution of grooming patterning, as highly stressed mice spend significantly more time grooming rostral areas than caudal *(8, 9)*. For example, data may be collected based on five anatomic areas (forepaws, head, body, hind legs, and tail/genitals) or simply a rostral (forepaw and head) versus caudal (body, legs, and tail/genitals) partition. Again, this distribution criterion may be modified or simplified to fit the individual needs of the researcher; however, maintaining a consistent standard within the laboratory will help prevent inaccuracies. Researchers may also classify each grooming bout as being directed to a single anatomic region or multiple regions, and calculate the percentage of grooming bouts and the percentage of time spent grooming for each category. Furthermore, the percentage of total grooming patterns, the percentage of time spent grooming, and the number of interruptions for each anatomic area may be assessed. Stressed anxious mice generally tend to display a greater number of interruptions, especially in rostral areas, when licking the forepaws or washing the face.

5. Typical/ Anticipated Results

A typical experiment assessing mouse grooming sensitivity to different pharmacological manipulations is presented in **Fig. 2.2**. In this study, anxiolytic drug diazepam normalized grooming patterning by lowering the percentage of incorrect transitions and interrupted bouts. In contrast, an anxiogenic substance pentylenetetrazole typically increased these indices and also increased the duration of grooming (*see (27)* for details). These data parallel recent data in rats showing that their grooming sequencing is sensitive to different classes of psychotropic drugs *(19, 24, 26)*.

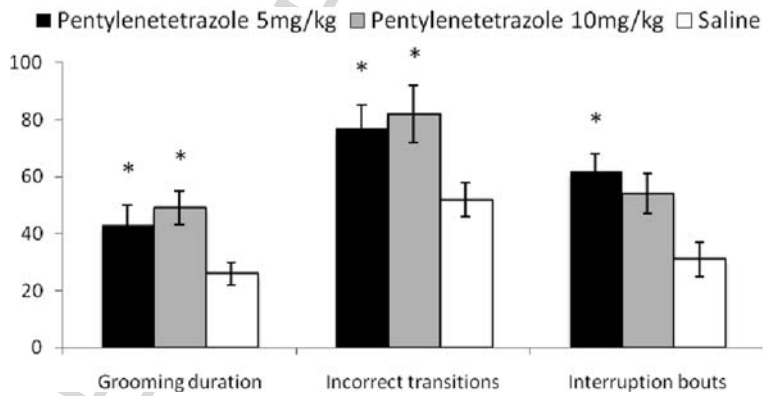

Fig. 2.2. Sensitivity of mouse grooming behaviors to anxiolytic and anxiogenic drugs *(27)*. Anxiolytic diazepam lowers the percentages of incorrect transitions and incorrect bouts, while anxiogenic drug pentylenetetrazole increases duration of grooming, with higher percentages of incorrect transitions and interrupted bouts. (*P $<$ 0.05, *U*-test).

Another typical experiment examining the regional distribution of mouse grooming is shown in **Fig. 2.3**, using the vitamin D receptor knockout mice as a model *(6)*. Note the difference between grooming behavior in the wild type and "anxious" mutant mice (i.e., more rostral grooming, less caudal grooming). Also notice the variances between the two different types of grooming: spontaneous (novelty-induced) and artificial (swim-induced) grooming mentioned above. Overall, while spontaneous grooming showed sensitivity to genetic differences, in this experimental model, the "more rigid" swim-induced grooming was not altered between the genotypes.

It is expected that analyses of mouse grooming behavior using microstructure-oriented approaches (**Table 2.1**) may be useful in examining rodent stress levels in experimental conditions *(6, 8, 9, 24, 26, 29, 30)*. Since grooming patterning in mice appears to be sensitive to stressful manipulations and could serve

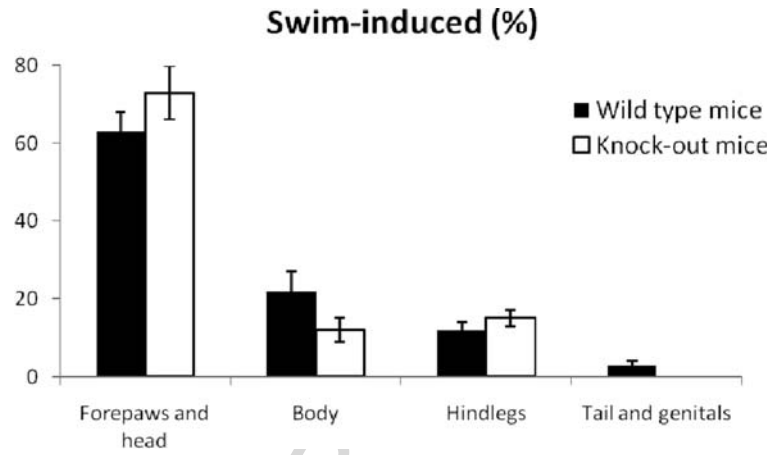

Fig. 2.3. Regional distribution of grooming patterns (of total taken as 100%) in the wild type and the vitamin D receptor knockout mice *(6)*. In the spontaneous novelty induced grooming test, the knockout mice displayed significantly higher percentages of forepaw, head and hind leg grooming, also showing less caudal (tail, genital) grooming than wild-type mice (*$P < 0.05$, *U*-test). Artificial swim-induced grooming showed no genotype differences between the groups.

as an additional measure of stress and anxiety, emotionality-related behaviors in mice could be investigated and assessed more accurately. Additionally, when paired with in-depth assessment of non-grooming phenotypes, grooming analyses could further confirm or invalidate unclear results.

New reliable methods for phenotyping mouse behavior could be formulated based on sensitivity of grooming analysis to alterations in patterning between various strains of mice. Researchers would also have a new useful criterion for choosing appropriate experimental subjects for their studies, since grooming (in addition to other specific phenotypes) could aid in the correct classification of novel strains of mutant or transgenic mice. Finally, mouse grooming behavior may also have a significant application in the study of human brain disorders *(10, 13, 44, 46)*. Likewise, brain lesion studies, particularly those focusing on basal ganglia motor control and patterned behavior regulation, could also lead to interesting neurobehavioral mouse models based on grooming activity and its patterning *(8)*.

6. Troubleshooting

Several practical recommendations, summarized here, may help the researchers to obtain more reliable and reproducible behavioral data.

1. If mice display abnormally high or low levels of grooming, it may be a strain-specific phenomenon *(28)*. While it is encouraged to further investigate strain differences, the researchers may need to re-assess the strain's suitability for their experiment.
2. Ameliorating the environmental and testing conditions would also aid in normalizing mice behaviors. This includes proper handling, a better enrichment, the use of fewer and/or less stressful tests, and improving husbandry *(8)*. If grooming activity remains too low, extending the tests for 5–10 more minutes may be a good practical solution, as it minimizes the initial anxiety and disinhibits grooming activity.
3. Factors such as altered skin/pain sensitivity and motor coordination deficits can be very pronounced in some mice. These factors may non-specifically alter animal behavior in a way that could be misinterpreted as altered grooming phenotype. To address this possibility and rule out non-specific factors, a careful examination of mouse neurological and sensory phenotypes is recommended.
4. When assessing the coat state, note that some mouse strains are poor (e.g., BALB/c mice) or excellent (e.g., A/J mice) groomers regardless of the level of their stress. Therefore, it is important to understand that, due to floor or ceiling effects, not every strain will produce reliable results in this test. Likewise, for socially housed mice, hetero-grooming may compensate for poor self-grooming, so the coat will have a clean appearance. To rule out this possibility, single housing may be employed (but with caution, since isolation itself may also have some behavioral effects).
5. When using novelty-induced grooming protocol, the size of arena (see above) is a very important factor. Since strain differences in anxiety and activity may affect all other behaviors, including grooming, the general rule is that the observation box needs to be relatively small. In a smaller box, the animals become familiar with the novelty faster, and this may help quickly reduce anxiety, enabling the mice to better "reveal" their grooming phenotypes.
6. Since it can be difficult to accurately detect exact grooming behaviors in mice, a frame-by-frame analysis with an event recorder is recommended. For example, without intense scrutiny of the animal's behavior, a stroke could easily be overlooked and the sequence could be misinterpreted. Video recording of all behavioral experiments is strongly recommended for more accurate grooming phenotyping.
7. Mice often engage in context-specific grooming (e.g., genital licking during mating, wound-inflicted body scratching) and, therefore, separate documentation of these instances may be

necessary *(8)*. Since mice may partake in both self-grooming and hetero-grooming behaviors, the researchers are advised to analyze these categories carefully. For example, in some mouse strains, hetero-grooming may naturally occur more frequently or for a longer duration, and consequently, self-grooming will be reciprocally decreased, which could be interpreted incorrectly as a stress-related response. It is useful to consider each occurrence separately, to avoid confounding data (e.g., reciprocal decrease in self-grooming in mice with abnormally increased hetero-grooming).

8. Rare "atypical" forms of grooming may also be difficult to categorize *(8)*. For example, some mice may partake in peculiar "pre-grooming" or "vertical grooming" *(28)* behaviors that could also lead to data misinterpretation. Thus, a careful analysis of both common and rare grooming activities is a key for accurate data collection and behavioral interpretation. In some other cases, grooming behavior needs to be separated from barbering (behavior-associated hair loss) phenotypes. This interesting rodent behavior will not be discussed here, but readers are encouraged to peruse recent works on this topic [e.g., *(10, 58–61)*]. Although separating self-grooming from hetero-barbering may be easy in most cases, self-grooming and self-barbering behaviors may sometimes be very similar.
9. In some instances, when using swim-evoked grooming models, the separation of swim test effects on artificial grooming per se and fatigability is necessary. To help differentiate between the two factors, researchers may shorten the swim test. For example, a 5-min swim test could potentially affect both artificial grooming and fatigability, whereas a very short 10-s swim session will only induce artificial grooming. Alternatively, using a different type of inductor that cannot evoke fatigue, such as smearing the animal with food, may be recommended to stimulate the artificial grooming.
10. Since the procedure that induces grooming may represent a stress for the mice, especially for some anxious mouse strains, it may be necessary to separate the procedure stress effects on grooming from those produced by artificial grooming inductors. Although this is a difficult task, some behavioral methods may enable dissection of spontaneous from artificial grooming. For example, while novelty stress-evoked grooming will habituate, artificial grooming is unlikely to decrease with repeated exposure. Likewise, artificial grooming microstructure will generally be more rigid and inflexible, compared to the spontaneous stress-evoked grooming.

7. Conclusion

Overall, there are clear benefits of in-depth analyses of mouse grooming activity and patterning in neurobiological experiments. First, it allows assessment of strain differences in grooming behaviors per se. Second, grooming activity and its sequencing may reflect fine differences in other domains, such as activity, motor patterning, anxiety, and depression. Finally, given the sensitivity of mouse grooming and its sequencing to various pharmacological and physiological manipulations, ethologically oriented analysis of grooming may be used extensively in pharmacogenetics and neurophysiology (e.g., for testing psychotropic drugs in different strains or for dissection of brain substrates involved in the regulation of behaviors). On the whole, behavioral analysis of mouse grooming can be a rich source of information in neuroscience and the biological psychiatry of anxiety and depression. Providing more comprehensive coverage of mouse behavioral phenotypes and offering ideas on their grooming peculiarities may assist researchers in correct data interpretation and selection of appropriate mouse models for their studies.

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Chapter 2

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AQ1	96	We have shortend the Running Head. Please Check.
AQ2	660	Please update.
AQ3	735	Please update.